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Salmonella in Dairy Operations in the United States: Prevalence and Antimicrobial Drug Susceptibility

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ABSTRACT

Salmonella serotypes are important foodborne pathogens of humans that can be acquired through consumption of contaminated meat and dairy products. *Salmonella* infection also can be a significant animal health issue. As part of a national study of U.S. dairy operations conducted between March and September 2002, fecal samples were collected from representative cows in 97 dairy herds in 21 states and were cultured to determine the prevalence of *Salmonella* shedding. *Salmonella* was recovered from the feces of at least one cow in 30.9% of the herds. Overall, 7.3% of fecal samples were culture positive for *Salmonella.* The three most frequently recovered serotypes were *Salmonella* Meleagridis (24.1%), *Salmonella* Montevideo (11.9%), and *Salmonella* Typhimurium (9.9%). The susceptibilities of *Salmonella* isolates recovered were determined using a panel of 16 antimicrobial drugs. *Salmonella* isolates recovered from dairy cows had relatively little resistance to these antimicrobial agents; 83.0% of the isolates were susceptible to all antimicrobials tested. This study provides updated information on the prevalence and susceptibility patterns of *Salmonella* in dairy herds and on cow and herd characteristics. These data contribute to our understanding of the ecology of *Salmonella* in the dairy farm environment.

Salmonella is pathogenic in many animal species including humans where it may cause foodborne illness. In 2002, there were 16,580 reported cases of laboratory-diagnosed foodborne illnesses caused by 10 organisms under surveillance by the Centers for Disease Control and Prevention (CDC). *Salmonella* was the most common bacterial pathogen identified, accounting for 36.4% of these cases *(3).* In a CDC analysis of the impact of food-related illnesses, *Salmonella* accounted for 25.6% of hospitalizations and 30.6% of deaths due to known foodborne pathogens *(15).*

Transmission of *Salmonella* organisms from dairy cattle to humans can occur through several routes, including consumption of contaminated milk, consumption of contaminated ground beef, and transmission of organisms by direct contact with feces *(10, 18, 20).* On-farm management practices can help control transmission of foodborne pathogens in dairy cattle. Initial quarantine of new animals, proper nutrient management, good hygiene, and access to fresh clean water are some of the practices that can reduce the possibility of introducing or increasing the prevalence of *Salmonella* within a dairy herd *(2, 4, 13, 14, 24).* Another important factor in transmission of this pathogen is exposure to contaminated feed. Some researchers have described the role of feedstuffs as a means of transmitting *Salmonella* to cattle *(5, 8, 12).* Reducing and controlling pathogens within herds may reduce the risk of human exposure and increase the health and productivity of dairy cows.

Although most *Salmonella* infections in cattle are subclinical, salmonellosis, the clinical disease caused by *Salmonella* infection, can present with fever, diarrhea, and occasionally death. The disease usually affects young calves at about 2 weeks of age, but adult animals also can exhibit clinical signs. Transmission of *Salmonella* is usually through the fecal-oral route, but young calves can be exposed through the umbilical cord *(17).* Morbidity and mortality can be significant and on rare occasions approach 100% within a herd. Salmonellosis can be a significant cause of financial and production losses on dairies *(16).*

In addition to the financial and production problems caused by salmonellosis, there is also growing concern regarding the acquisition of antimicrobial drug resistance determinants among specific bacterial pathogens such as *Salmonella.* Of particular concern are the resistance patterns of zoonotic bacteria and how resistance may affect human health. Antimicrobial drug–resistant *Salmonella* strains have been associated with increased morbidity and mortality in humans *(9).* The objectives of this study were to investigate the prevalence of *Salmonella* in U.S. dairy herds, to evaluate management factors associated with fecal shedding of *Salmonella,* and to monitor antimicrobial drug susceptibility of *Salmonella* isolates recovered from dairy operations.

MATERIALS AND METHODS

Study population and sample collection. A stratified random sample of dairies was chosen from the U.S. Department of

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Agriculture (USDA) National Agricultural Statistics Service listing for each of 21 selected states from four regions of the United States. Regional categories for analysis included the west (California, Colorado, Idaho, New Mexico, Texas, and Washington), midwest (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, and Wisconsin), northeast (New York, Pennsylvania, and Vermont), and southeast (Florida, Kentucky, Tennessee, and Virginia). This sample represented 85.5% of U.S. dairy cows and was the basis for selecting herds for participation in the USDA Animal and Plant Health Inspection Service National Animal Health Monitoring System (NAHMS) Dairy 2002 study. Participation in the study included over 2,400 dairy producers in the first phase and 1,000 producers in the second phase *(22).* A convenience sample of 100 of these dairies was selected for fecal sampling. The number of dairies included was limited because of laboratory capacity, and approximately five operations per state were selected. Previous history of illness consistent with salmonellosis was not a selection factor.

Dairies were visited between 27 March and 25 September 2002. Data were collected on herd and animal health management factors via interviews with producers using a standardized questionnaire *(22).* Twenty-four to 40 fecal samples were collected from milking cows 2 years of age or older via rectal retrieval. A separate glove was used to collect each fecal sample to avoid cross-contamination during sampling. Samples were placed in Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) and shipped on ice to the USDA Agricultural Research Service Antimicrobial Resistance Research Unit (ARRU) in Athens, Ga., for culturing and susceptibility testing.

Bacteriologic culture and antimicrobial susceptibility methods. *Salmonella* isolates were cultured as previously described *(25).* Approximately 1 g of feces from each sample was placed into each of two culture media, gram-negative Hajna broth and tetrathionate broth, which were incubated at 37°C for 24 and 48 h, respectively. Following primary enrichments, 100-μl culture aliquots were transferred into Rappaport R-10 medium for secondary enrichment. In each case, the Rappaport R-10 medium was incubated overnight at 37°C and then streaked onto brilliant green agar with sulfadiazine and xylosine-lysine-tergitol-4 plates. All plates were incubated overnight at 37°C. From each sample, as many as four colonies with the typical appearance of *Salmonella* were inoculated into triple sugar iron and lysine iron agar slants. All slants were incubated overnight at 37°C. Isolates presumed to be *Salmonella* were serogrouped using serogroup-specific sera (BD Diagnostic Systems, Sparks, Md.) and sent to the National Veterinary Services Laboratories (Ames, Iowa) for serotyping.

Each distinct *Salmonella* isolate was also submitted to the ARRU for antimicrobial drug susceptibility testing as part of the National Antimicrobial Resistance Monitoring System—Enteric Bacteria (NARMS). NARMS is a national collaboration of the USDA, the Food and Drug Administration, and the CDC to monitor trends in antimicrobial drug resistance over time. Susceptibility testing was conducted with a custom-designed NARMS panel of 16 antimicrobial drugs using a Sensititre semiautomated testing system (TREK Diagnostic Systems, Inc., Cleveland, Ohio). Antimicrobial drugs tested in 2002 were amikacin (Am), amoxicillin–clavulanic acid (Amo), ampicillin (Amp), cefoxitin (Cefo), ceftiofur (Ceft), ceftriaxone (Ceftri), cephalothin (Ceph), chloramphenicol (Chlor), ciprofloxacin (Cip), gentamicin (Gen), kanamycin (Kan), nalidixic acid (Nal), streptomycin (Str), sulfamethoxazole (Sulf), tetracycline (Tet), and trimethoprim-sulfamethoxazole (Tris). The MIC for each isolate was determined, and each isolate was classified as susceptible, intermediate, or resistant according to guidelines published by the National Committee on Clinical Laboratory Standards for broth-microdilution susceptibility testing, when available. Otherwise, breakpoint interpretations were determined using NARMS guidelines *(7).*

TABLE 1. *Number (%) of dairy herds by size and region*

Region	Values by herd size[a]			Total
	Small	Medium	Large	
West	2 (2.1)	8 (8.2)	18 (18.6)	28 (28.9)
Midwest	19 (19.6)	18 (18.6)	3 (3.1)	40 (41.2)
Northeast	8 (8.2)	5 (5.2)	2 (2.1)	15 (15.5)
Southeast	4 (4.1)	8 (8.2)	2 (2.1)	14 (14.4)
Total	33 (34.0)	39 (40.2)	25 (25.8)	97

[a] Small, <100 head; medium, 100–500 head; large, >500 head.

Statistical analysis. A herd was considered positive for *Salmonella* when at least one fecal sample was positive. Sample-level and operation-level comparisons were performed using chi-square tests. For sample data, the analyses accounted for the clustering of samples by operation.

RESULTS AND DISCUSSION

Dairy operations included in the survey. Fecal samples from 97 dairy operations were collected and cultured for *Salmonella* species. Table 1 includes information on herd size and geographical location. Overall, 34.0% of the dairy operations were classified as small (<100 cows), 40.2% were medium (100 to 500 cows), and 25.8% were large (>500 cows). Even though these dairy operations were a convenience sample of dairies across the United States, they reflect the diversity of regions and herd sizes present in the U.S. dairy population *(21).*

Prevalence of *Salmonella.* Of the 3,709 fecal samples that were collected and cultured, 269 (7.3%) were positive for *Salmonella.* This prevalence is slightly higher than that in the NAHMS Dairy 1996 study, in which 5.4% of all milkcows sampled were shedding *Salmonella (25).* Seasonality may be a factor in the difference in prevalence between these two studies *(1, 11, 19).* In the 1996 study, the samples were collected between February and July, whereas the samples in the present study were collected in the warmer months of March through September. The prevalence of *Salmonella* in the present study was highest in September, when 18% of the samples were positive ($P < 0.001$). The sample prevalence of *Salmonella* also varied by region ($P = 0.0016$). Of the *Salmonella*-positive cows, 49.4% (133 of 269) were from the west, 35.3% (95 of 269) were from the midwest, 13.8% (37 of 269) were from the southeast, and only 1.5% (4 of 269) were from the northeast.

From the 269 positive fecal samples, 294 discrete *Salmonella* isolates were identified, and 24 fecal samples yielded more than one serotype (23 with two serotypes and 1 with three serotypes). These data suggest that some animals may be shedding multiple strains at the same time. Serogroup E was the most commonly observed serogroup,

TABLE 2. Salmonella *isolates from dairy operations by serotype*

Serogroup	Serotype	Isolates		Dairy herds	
		No.	%	No.	%[a]
B		57	19.4	9	9.3
	Agona	22	7.5	3	3.1
	Kiambu	1	0.3	1	1.0
	Reading	1	0.3	1	1.0
	San Diego	3	1.0	1	1.0
	Typhimurium	29	9.9	3	3.1
	Untypeable	1	0.3	1	1.0
C1		73	24.8	14	14.4
	Hartford	1	0.3	1	1.0
	Infantis	3	1.0	1	1.0
	Livingstone	2	0.7	1	1.0
	Mbandaka	12	4.1	5	5.2
	Montevideo	35	11.9	8	8.3
	Ohio	12	4.1	1	1.0
	Oranienburg	2	0.7	1	1.0
	Tennessee	1	0.3	1	1.0
	Thompson	4	1.4	1	1.0
	Untypeable	1	0.3	1	1.0
C2		9	3.1	6	6.2
	Newport	8	2.7	5	5.2
	Untypeable	1	0.3	1	1.0
C3		28	9.5	8	8.2
	Kentucky	28	9.5	8	8.2
E		116	39.5	15	15.5
	Anatum	6	2.0	3	3.1
	Give	4	1.4	2	2.1
	Meleagridis	71	24.1	5	5.2
	Muenster	6	2.0	3	3.1
	Newington	5	1.7	1	1.0
	Senftenberg	12	4.1	4	4.1
	Uganda	1	0.3	1	1.0
	Untypeable	11	3.7	2	2.1
G		1	0.3	1	1.0
	Cubana	1	0.3	1	1.0
I		1	0.3	1	1.0
	Barranquilla	1	0.3	1	1.0
K		8	2.7	2	2.1
	Cerro	8	2.7	2	2.1
X		1	0.3	1	1.0
	Bergen	1	0.3	1	1.0

[a] Percentage of herds within each serogroup category will not add up with respect to the percentage of herds for the serotypes because in some herds multiple serotypes were isolated from one fecal sample.

comprising 39.5% of isolates originating from 15.5% of herds sampled (Table 2).

Twenty-eight different serotypes were identified. Of these, 62.9% of isolates were represented by five serotypes: *Salmonella* Meleagridis (24.1%), *Salmonella* Montevideo (11.9%), *Salmonella* Typhimurium (9.9%), *Salmonella* Kentucky (9.5%), and *Salmonella* Agona (7.5%) (Table 2). *Salmonella* Montevideo and *Salmonella* Kentucky were cultured from the largest number of herds, with each serotype isolated on 26.7% (8 of 30) of the dairy herds that had positive samples. *Salmonella* Kentucky was found predominantly in the southeast ($P < 0.0001$), whereas the other four most common serotypes were found primarily in the west and midwest ($P < 0.0001$) (Table 3). There was also a seasonal difference in prevalence among the five most common serotypes. *Salmonella* Typhimurium had a higher prevalence in the summer months (July through September), whereas the other four most common serotypes had a higher prevalence in the spring months (April through June) (data not shown). The prevalent serotypes differed slightly from those in the NAHMS Dairy 1996 study, in which *Salmonella* Montevideo, *Salmonella* Cerro, *Salmonella* Kentucky, *Salmonella* Menhaden, *Salmonella* Anatum, and *Salmonella* Meleagridis were the most frequent isolates recovered from milk cows on farms *(25).* In a recent related study, the most common serotypes isolated from bulk tank milk samples were *Salmonella* Montevideo, *Salmonella* Newport, *Salmonella* Muenster, *Salmonella* Meleagridis, and *Salmonella* Cerro *(23).* These serotypes differ from those of isolates recovered from clinical cases submitted to the National Veterinary Services Laboratory, where the most common serotypes reported are *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Montevideo, *Salmonella* Dublin, and *Salmonella* Uganda *(6).* The variety of serotypes in this and previous studies highlights the diversity and wide distribution of *Salmonella* serotypes in cattle.

TABLE 3. *Regional distribution of the five most common* Salmonella *serotypes in dairy herds*

Salmonella serotype	West	Midwest	Northeast	Southeast	Total
Meleagridis	33	38	0	0	71
Montevideo	29	4	0	2	35
Typhimurium	0	25	4	0	29
Kentucky	6	1	0	21	28
Agona	22	0	0	0	22

Cows sampled were presumed to be clinically healthy with no overt signs of diarrhea. There were no significant differences in fecal shedding of *Salmonella* among cows when analyzed by cow type (lactating versus dry), fecal score, lactation number, or body condition score. The majority of cows sampled were milking cows in their second or third lactation with normal body condition and fecal scores.

Culture-positive dairy operations. Overall, 30 of the 97 herds (30.9%) had at least one fecal culture that was positive for *Salmonella.* The herd prevalence is slightly higher, although not significantly so, than the prevalence found in the NAHMS Dairy 1996 study, in which 27.5% of U.S. dairy operations had at least one milk cow shedding *Salmonella (25).* This higher *Salmonella* prevalence may be a result of the current trend toward more concentrated, larger herd size operations or may be within the limits of normal annual variation. In the present study, an association was found between fecal shedding and herd size. Dairy operations with large herds (>500 cows) represented 43.3% ($P = 0.009$) of the farms that had at least one fecal culture positive for *Salmonella.* Large-herd operations had the

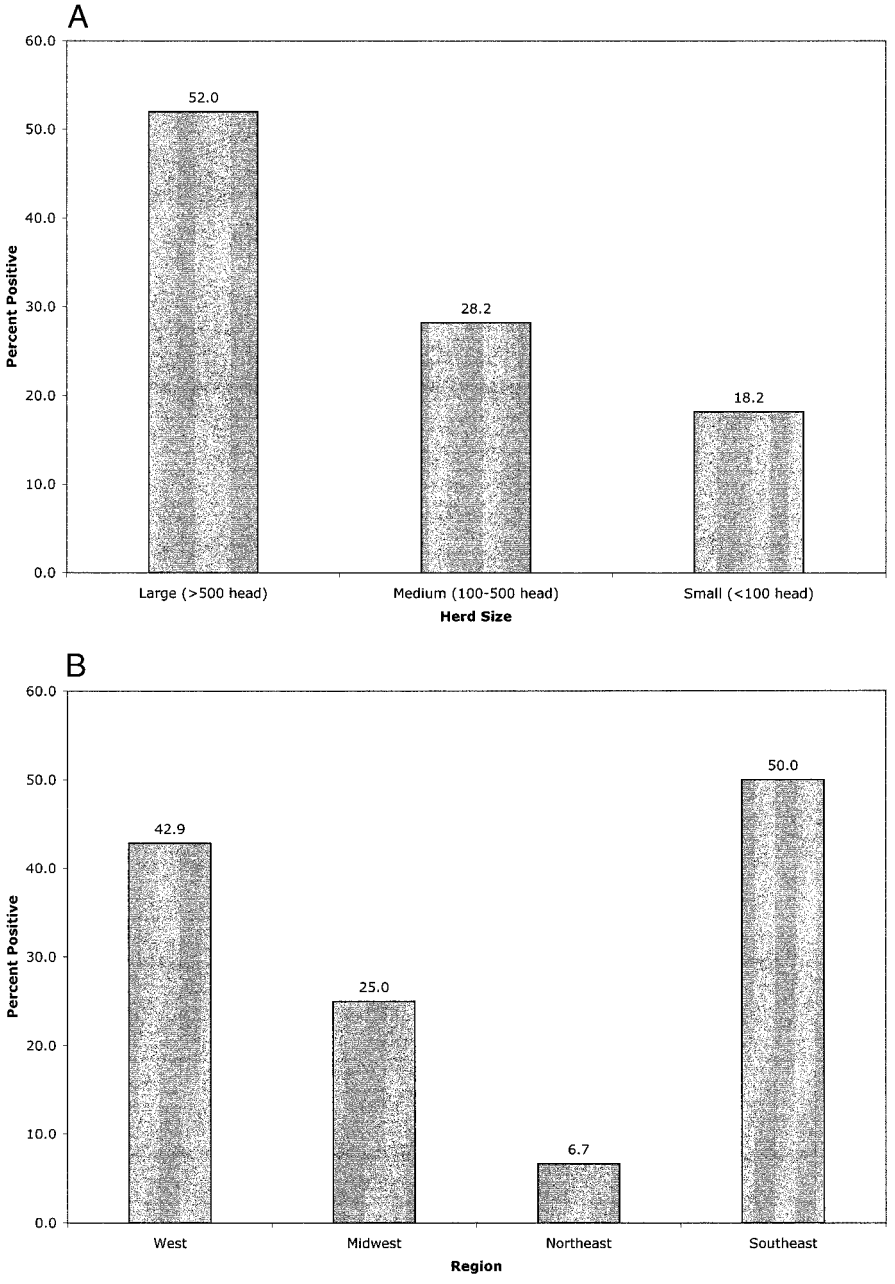

FIGURE 1. *Percentage of herds with at least one positive* Salmonella *sample by herd size and region. (A) Herd size classifications are large (>500 head), medium (100 to 500 head) and small (<100 head). (B) Region classification was assigned as described in ''Materials and Methods.''*

highest percentage of positive herds, at 52.0% (Fig. 1A). Large herds may require more intensive management practices. Crowding, contact, and stress in these operations may contribute to increased infection or shedding rates. Large-herd operations reported introducing more new animals to the herd compared with operations that include smaller herds. These new animals may be carriers of *Salmonella*, and introduction of a higher number of replacement animals may contribute to an increase in *Salmonella* infection. Within herds with one or more *Salmonella*-positive sample, an average of 23.0% of samples were culture positive. The range of prevalence within culture-positive herds was 2.5 to 97.4%, with a median of 10.0%. Although there was not a significant difference in herd prevalence by region overall, 73.3% (22 of 30) of the herds with at least one *Salmonella*-positive fecal sample were in the west and midwest. In the southeast, 50.0% (7 of 14) of herds sampled had at least one positive sample (Fig. 1B).

No association was found between reported cases of diarrhea within herds and *Salmonella*-positive fecal cultures. Of the 30 herds with positive fecal cultures, 26.7% (8 of 30) reported having no cases of diarrhea in any dairy cow during the previous year, suggesting subclinical infections. Of these eight positive herds, six had only one or two positive cows. There were no significant differences in serotypes recovered from operations with no reported diarrhea cases compared with operations that reported cows with diarrhea. Of the herds positive for *Salmonella* and reporting diarrhea cases during the previous year, 0.4 to 13.0% of cows experienced morbidity due to diarrhea. The within-herd prevalence of reported diarrhea cases in all herds, with or without *Salmonella*-positive fecal results, ranged from 0.0 to 75.0%. The subclinical infections within herds did not appear to have an overall impact on milk production as evaluated by rolling herd averages of pounds of milk produced. These herd averages were categorized as

TABLE 4. *Percentage of* Salmonella *isolates from dairy operations by level of resistance to various antimicrobials*

Antimicrobial	% susceptible	% intermediate	% resistant
Amikacin	100.0	0.0	0.0
Amoxicillin–clavulanic acid	95.2	0.0	4.8
Ampicillin	95.6	0.0	4.4
Cefoxitin	94.2	2.0	3.7
Ceftiofur	95.6	0.0	4.4
Cefriaxone	97.6	2.4	0.0
Cephalothin	94.9	0.3	4.8
Chloramphenicol	94.9	0.7	4.4
Ciprofloxacin	100.0	0.0	0.0
Gentamicin	99.3	0.0	0.7
Kanamycin	99.3	0.0	0.7
Nalidixic acid	100.0	0.0	0.0
Streptomycin	90.5	0.0	9.5
Sulfamethoxazole	96.3	0.0	3.7
Tetracycline	87.8	0.3	11.9
Trimethoprim-sulfamethoxazole	100.0	0.0	0.0

low (<16,000 lb per cow per year), medium (16,000 to 20,000 lb per cow per year), or high (>20,000 lb per cow per year). There was no significant association between rolling herd milk production averages and the recovery of *Salmonella* from fecal samples within herds ($P = 0.48$). Overall, 50.5% (49 of 97) of the herds reported high rolling herd milk production averages. However, two of the three *Salmonella*-positive operations that had rolling herd averages of less than 16,000 lb had the two highest within-herd *Salmonella* prevalences (97.5 and 82.1%).

Antimicrobial susceptibility. *Salmonella* isolates from dairy cows had relatively little resistance to a number of antimicrobial agents; 83.0% were susceptible to all antimicrobial drugs tested. All isolates were susceptible to amikacin, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole (Table 4). Overall, 12.2% of the isolates were resistant to at least one antimicrobial drug, and 4.8% were resistant to more than one antimicrobial drug. Resistance to tetracycline was most common (11.9% of all isolates, 35 of 294) followed by resistance to streptomycin (9.5%, 28 of 294). The patterns of resistance are described in Table 5. Resistance to tetracycline, cephalothin, and amoxicillin–clavulanic acid was observed in all multiresistant combinations. Resistance profiles for serotypes isolated in the study that were resistant to one or more antimicrobials are described in Table 6. Serotypes not listed were susceptible to all the antimicrobials tested. Multidrug resistance was more common among certain serotypes, including *Salmonella* Newport, *Salmonella* Reading, and *Salmonella* Typhimurium. Of isolates that were resistant to more than one antimicrobial drug, 50% (7 of 14) were *Salmonella* Newport. These isolates were found in four different herds. There is currently concern in the United States about the emergence of a strain of multidrug-resistant *Salmonella* Newport and the potential impact on human health *(9, 26)*. However, in the present study *Salmonella* Newport isolates represented only 2.7% (8 of 294) of the isolates recovered and were present on only 5.2% (5 of 97) of operations. The multidrug-resistant form was even less common, accounting for only 2.4% (7 of 294) of the isolates. One *Salmonella* Newport isolate was susceptible to all antimicrobial drugs tested. Four of the five most common serotypes did not show any multidrug resistance.

The results of this study indicate that the prevalence of *Salmonella* on dairy operations in the United States continues to remain relatively low, with 7.3% of cows and 30.9% of herds having one or more *Salmonella*-positive fecal culture. There has been little change in prevalence compared

TABLE 5. *Resistance patterns among 294* Salmonella *isolates from dairy operations*

Resistance[a]	No. (%) of *Salmonella* isolates
Susceptible to all antimicrobials	244 (83.0)
Tet	21 (7.1)
Str	15 (5.1)
Amo, Amp, Cefo, Ceft, Ceph, Chlor, Str, Sulf, Tet	7 (2.4)
Amo, Amp, Cefo, Ceft, Ceph, Chlor, Gen, Kan, Str, Sulf, Tet	2 (0.7)
Amo, Amp, Cefo, Ceft, Ceph, Chlor, Str, Tet	2 (0.7)
Amo, Amp, Ceft, Ceph, Chlor, Str, Sulf, Tet	2 (0.7)
Amo, Ceph, Tet	1 (0.3)
Total	294 (100.0)

[a] See ''Materials and Methods'' for definitions of abbreviations.

TABLE 6. *Resistance profiles of* Salmonella *serotypes resistant to one or more antimicrobials*

Serotype	No. of isolates	No. of antimicrobials serotype is resistant to	Resistance pattern	No. of herds
Agona	20	0	None	3
	2	1	Str	1
Anatum	4	0	None	2
	2	1	Tet	1
Kentucky	13	0	None	7
	15	1	Tet	2
Mbandaka	11	0	None	5
	1	3	Amo, Ceph, Tet	1
Montevideo	20	0	None	8
	12	1	Str	1
	3	1	Tet	1
Newport	1	0	None	1
	1	8	Amo, Amp, Cefo, Ceft, Ceph, Chlor, Str, Tet	1
	5	9	Amo, Amp, Cefo, Ceft, Ceph, Chlor, Str, Sulf, Tet	3
	1	11	Amo, Amp, Cefo, Ceft, Ceph, Chlor, Gen, Kan, Str, Sulf, Tet	1
Reading	1	11	Amo, Amp, Cefo, Ceft, Ceph, Chlor, Gen, Kan, Str, Sulf, Tet	1
Typhimurium	24	0	None	2
	1	8	Amo, Amp, Cefo, Celt, Ceph, Chlor, Str, Tet	1
	2	8	Amo, Amp, Ceft, Ceph, Chlor, Str, Sulf, Tet	2
	2	9	Amo, Amp, Cefo, Ceft, Ceph, Chlor, Str, Sulf, Tet	2
Untypeable	12	0	None	1
	1	1	Str	1
	1	1	Tet	1

with that in a similar study conducted in 1996. In the present study, a higher prevalence of *Salmonella* occurred during the later months of summer and in the western region of the country. In the United States, resistance of *Salmonella* on dairy operations to antimicrobial drugs also continues to be low. However, the predominant *Salmonella* serotypes tend to change over time and multidrug-resistant strains vary.

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